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# Terahertz identification and quantification of penicillamine enantiomers

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**Abstract** Identification and characterization of *L*-, *D*- and *DL*- penicillamine were demonstrated by Terahertz time-domain spectroscopy (THz-TDS). To understand the physical origins of the low frequency resonant modes, the density functional theory (DFT) was adopted for theoretical calculation. It was found that the collective THz frequency motions were decided by the intramolecular and intermolecular hydrogen bond interactions. Moreover, the quantification of penicillamine enantiomers mixture was demonstrated by a THz spectra fitting method with a relative error of less than 3.5%. This technique can be a valuable tool for the discrimination and quantification of chiral drugs in pharmaceutical industry.

**Key words** Terahertz spectroscopy, Vibration modes, Quantitative analysis



## 1 Introduction

Terahertz (THz) time-domain spectroscopy (TDS) is an extremely promising technique for chemical and biomedical applications since rotational and vibrational transitions of molecules and the low frequency vibration of crystal lattice lie within the far- and mid-infrared spectral range[1]. At present, a wide range of applications of THz-TDS technology have been reported[2-7]. Numerous structural biomolecules and chemicals have been investigated and the results indicate that the absorption characteristics in THz range are directly related to the composition and structure of molecules. The highly-sensitive THz spectroscopy provides us with a particular fingerprint method to discriminate molecules.

At present, there is an increasing interest in studying low frequency vibration modes in chiral drugs because these modes can be used to identify isomers. The recent trend in pharmaceutical industry is to market the drugs in a pure enantiomeric form because different chiral forms of a drug may have different biological properties and clinical effect[8]. For some drugs, one kind of isomer is biologically-active and effective for therapy, while another has no effect or even toxicity. Hydrogen bonding and van der waals force play important roles in the interaction between drugs and organism systems. The corresponding vibrational modes of these weak interaction forces usually lie in THz range, which provides the theoretical basis for using THz technology to achieve molecular discrimination and study their interactions.

Penicillamine is a thiol drug used in the treatment of rheumatoid arthritis. Only pure *D*-penicillamine is used in clinic since the *L*- form and *DL*-racemate are much more toxic, as shown by severe adverse reactions such as neuritis in patients treated with the *DL*-penicillamine[9]. A high degree of purification of *D*-penicillamine is essential for drug products. The X-ray crystal structure and IR spectra of a racemic mixture of *D*- and *L*- penicillamine have been studied. However, there is a lack of study on their vibrational modes[10]. In this paper, the low frequency absorption spectra of *L*-, *D*- and *DL*- Penicillamine are

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measured and characterized by THz-TDS. DFT theoretical calculations are carried out at the B3LYP/6-311G** level using Gaussian 03 packages and Gauss-View visualization program[11]. Excellent agreement with experiments is achieved in the values of calculated vibrational mode frequencies. In addition, quantification of penicillamine enantiomers in the mixtures is determined by fitting the obtained absorption THz spectra.

## 2 Experimental

### 2.1 Setup

The setup for THz-TDS system is the same as the one described in detail in Ref.[12]. The whole system is placed in a closed box purged with dry nitrogen gas in order to minimize the absorption of water vapor. The setup allows spectroscopic data to be recorded in the frequency range from 200 GHz to 2.2 THz with a dynamic range of about 1000 and a spectral resolution of better than 40 GHz. The measurements are considered accurate between 0.2 and 1.9 THz because of the excessive attenuation caused by the samples at higher frequency.

### 2.2 Data analysis

The method detailed in the paper by Duvillaret *et al.*[13] is used to extract the complex refractive index from the THz-TDS data. In short, the extraction of parameters is performed in two steps. Firstly, a reference spectrum is obtained in the absence of the sample, and then a spectrum in the presence of the sample is measured. The ratio of the sample data to the reference data is the complex transmission coefficient, which is a function of frequency. As noted in Ref.13, the error function is gradually minimized using a standard algorithm, which yields the desired values for complex refractive index.

### 2.3 Sample preparation

All samples were crystalline powder with purity higher than 98%, purchased from Sigma-Aldrich. Fig.1 shows the molecular structures of *L*-, *D*- and *DL*-penicillamine. All samples were carefully mixed with polyethylene (PE) powder with a weight ratio of 1:1 and a total weight of 200 mg, and then pressed into 1.554−1.642 mm thick pellets. PE was chosen because of low absorption coefficients (<5 $cm^{-1}$ below 4 THz).

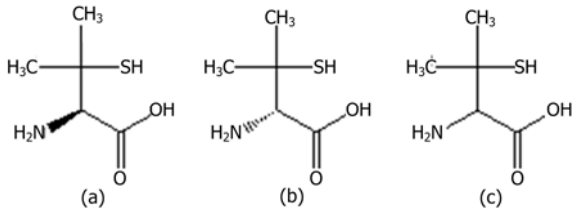


**Fig.1** Molecular structures of (a) *L*-, (b) *D*- and (c) *DL*-penicillamine.

### 2.4 Computational methods

Theoretical calculation was performed by density functional theory (DFT). DFT method is widely used in molecular spectra calculations. The amount of its computation is considerably smaller than HF (Hartree-Fock) and MP2 (Moller-Plesset) methods under the same conditions, and its requirements are relatively low for computer system performance. Moreover, the DFT method can obtain more accurate calculation results relative to the HF method because of considering the electron correlation energy[14]. Geometry optimization and frequency analysis of the title compounds were performed using DFT B3LYP with 6-311++G** basis set[15,16]. The chiral penicillamine and their racemate exist with a zwitterion structure in the solid state. The two C-O bond lengths in carboxylate group are equal and three hydrogen atoms are attached to a nitrogen atom[10]. The X-ray structure of racemate *DL*-penicillamine provides useful information of the interaction between the enantiomers. The isolated and dimer models of *L*- and *D*-penicillamine were also investigated.

## 3 Results and discussion

The absorption spectra of *L*-, *D*- and *DL*-penicillamine measured at room temperature are shown in Fig.2. The experimental results show significant difference in the absorption spectra of enantiomers (*L*- and *D*-penicillamine) and their racemate (*DL*-penicillamine). The absorption spectra of *L*- and *D*-penicillamine also show some differences in THz range. *L*- penicillamine has two absorption peaks located at 1.52 and 1.88 THz, while the peaks lie at 1.58 and 1.87 THz for *D*-penicillamine. This is in good agreement with the Raman spectrum of

*D*-penicillamine measured by Howard-lock H E, *et al*[10]. In addition, two weak absorption peaks appear at 1.00 and 1.32 THz for *D*-penicillamine. The crystal structures of enantiomers (*L*- and *D*-penicillamine) are orthorhombic with the space group $P222_1$, and *DL*-penicillamine is monoclinic with the space group $P2_1/c$[10]. It indicates that THz-TDS is sensitive to the change of the crystal structure and can be used for identification application.

The vibrational frequencies were calculated by quantum chemical theory using the packages of Gaussion 03. The molecular structures of *L*-, *D*- and *DL*-penicillamine were made through geometrical optimization. No negative frequency mode was found in the results of calculation. Table 1 shows the experimental modes and the calculated modes values at B3LYP/6-311++G** level for *L*-, *D*-, *DL*-penicillamine in 0.2−2.0 THz range. Fewer frequency modes were obtained from single molecule model compared with the dimer and racemic molecules models. It suggests that the molecular interactions are necessary to be considered in calculating low frequency vibration.

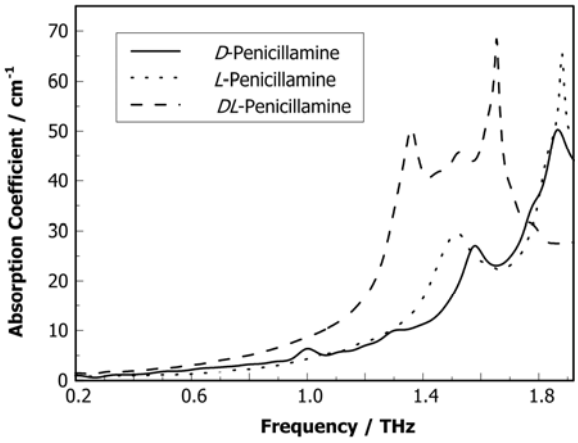


**Fig.2** THz absorption spectra of *L*-, *D*- and *DL*-penicillamine.

**Table 1** Observed and calculated vibrational mode frequencies for *L*-, D- and DL-penicillamine in 0.2–2.0 THz region

| Sample | Exp./THz | Ref.[10] | B3LYP/6-311++G** | | Approximate Vibrational Mode Assignment |
|---|---|---|---|---|---|
| | | | Single | Dimer (IR intensity) | |
| *L*- | | | | 0.50 (2.6857) | Butterfly (face to face) |
| | | | | 1.05 (6.57) | Torsion |
| | | | | 1.20 (3.2327) | Wiggle |
| | | | | 1.39 (0.1632) | Torsion |
| | 1.52 | | 1.61 | – | |
| | 1.88 | | | 1.90 (1.3298) | Torsion |
| *D*- | | | | 0.49 (2.7132) | Butterfly (face to face) |
| | 1.00 | | | 1.04 (6.4357) | Torsion |
| | | | | 1.19 (3.3303) | Wiggle |
| | 1.32 | | | 1.38 (0.1759) | Torsion |
| | 1.58 | 1.56 | 1.61 | – | |
| | 1.87 | 1.80 | | 1.89 (1.2677) | Torsion |
| *DL*- | | | | 0.63 (1.42) | Butterfly (face to face) |
| | 1.36 | | | 1.29 (0.00) | Wiggle |
| | 1.53 | | | 1.41 (3.65) | Torsion |
| | 1.65 | | 1.61 | 1.67 (2.20) | Torsion |

According to the calculation by Gauss-View visualization program, approximate structural and low frequency vibrational modes were suggested. Two hydrogen atoms of the protonated amine take part in the formation of intra- and intermolecular hydrogen bonds. Two strong intermolecular hydrogen bonds N-H···O=C (0.152 nm) with N···O distance equal to 0.259 nm exist in a pair of racemate DL-penicillamine molecules. These interactions play an important role in maintaining the molecular structure in the solid state. The corresponding modes of racemate *DL*-penicillamine are shown in Fig.3. The DFT calculation predicts four bands at 0.63, 1.29, 1.41 and 1.67 THz for *DL*-penicillamine. The vibrational mode at 0.63 THz is assigned as butterfly mode with face to face of *D*- and *L*-molecules of penicillamine. The mode at 1.29 THz comes from wiggle and modes at 1.41 and 1.67 THz are regarded as torsions which cause weak deformation vibrations of whole molecules. Therefore, the intra- and intermolecular hydrogen bonds are

involved in the molecular motion. The low frequency modes obtained from the theoretical calculation indicate that the vibration modes are not localized the atomic motions but the whole molecular motions.

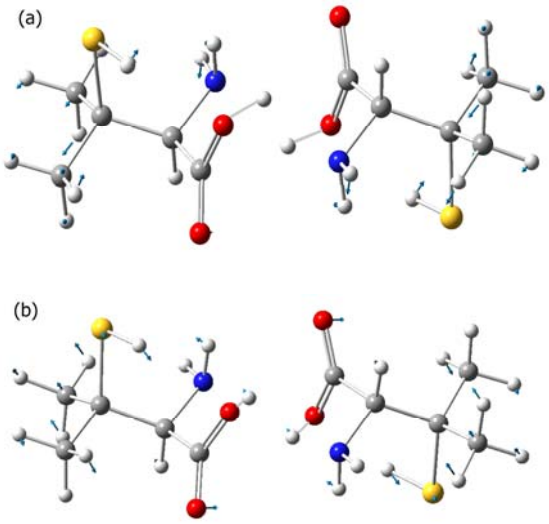


**Fig.3** Different vibrational modes of racemate DL-penicillamine in 1.41 THz (a) and 1.67 THz (b). Atomic color codes: gray (carbon), white (hydrogen), blue (nitrogen), red (oxygen) and yellow (sulfur).

When the dimer model is adopted to simulate the low frequency motion of *L*-, *D*- and *DL*- molecules, there are some differences of vibrational frequency between racemate and its enantiomers, which is caused by the different crystal structures of racemate (monoclinic $P2_1/c$) and the enantiomer (orthorhombic $P222_1$). In addition, the vibrational frequencies of the enantiomers in *L*- and *D*- molecules are somewhat different. This can be understood from the following discussion. On one hand, the enantiomers have the same crystal structure parameters except the configuration, which would form different hydrogen bonds or relative motions with their respective adjacent molecules. It suggests that the frequency difference of penicillamine enantiomers obtained by experiment originates from their different configurations. On the other hand, theoretical model does not simulate the actual experimental condition perfectly and gives almost the same vibrational frequencies for *L*- and *D*-penicillamine. For example, multiple molecular interactions are not considered. This causes some discrepancy between the theoretical prediction and experimental results, such as in the value of peak frequency.

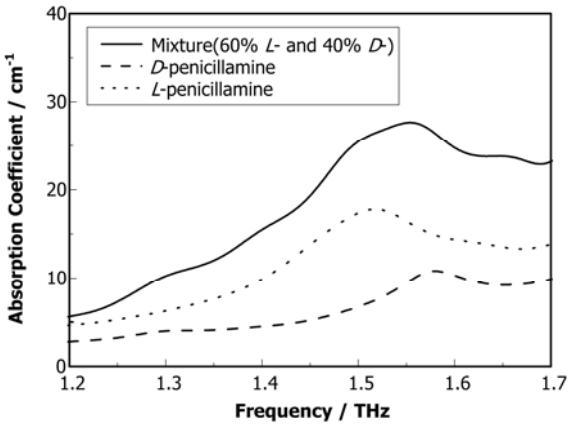


**Fig.4** Absorption spectra of *L*-, *D*-penicillamine and their mixture ( 60% *L*- and 40% *D*- ).

To demonstrate the capability of quantification using THz-TDS, we measured the absorption spectra of solid powder mixtures of *L*- and *D*-penicillamine with different weight ratio. The weight content of *L*-penicillamine in the mixtures was 40%, 60% and 80%, respectively. Fig.4 shows the absorption spectra of *L*-, *D*-penicillamine and their mixture (60% *L*- and 40% *D*-). According to the Lambert-Beer law, if the absorption spectra of pure sample and their mixture are measured, the relative percentage of pure sample in the mixture can be obtained using the least square fit method. In this work, the absorption spectra of the penicillamine enantiomers and their mixture were fitted in the range from 1.2 to 1.7 THz, and the relative content of penicillamine enantiomers was obtained. A series of mixtures of penicillamine enantiomers with different weight ratio were measured and analyzed. The calculated weight percentage concentrations of *L*-penicillamine in the mixture are 40.9%, 62.1% and 81.5%, which are quite close to the actual concentrations of 40%, 60% and 80%. The analytic errors are 2.5%, 3.5% and 1.9%, respectively. The concentration of penicillamine enantiomers in their mixture can be quantitatively determined using the THz spectra fitting method. This provides a valuable tool for quantitative analysis of medicine and potential applications in pharmaceutical industry.

## 4 Conclusion

We have characterized the far infrared absorption properties of *L*-, *D*- and *DL*-penicillamine by

THz-TDS in the region of 0.2-1.9 THz. The chiral molecules show distinct THz fingerprints due to their different crystal structure and molecular conformation. The DFT theory with the 6-311++G** basis set has been employed to calculate the low frequency resonance. More accurate information has been obtained from the dimer model than from the isolated molecule model, which suggests that weak molecular interaction needs to be taken into account in calculating low frequency vibration. The calculation results indicate that the characteristic vibrational frequencies come from various collective motions and both intra- and inter-molecular hydrogen bond interactions are involved. The quantification of penicillamine enantiomers mixture has been achieved by the THz spectra fitting method with a relative error less than 3.5%. The study shows that THz-TDS is a capable tool for qualitative and quantitative detection of chiral drugs.

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